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Factors Driving the Abundance of *Ixodes ricinus* Ticks and the Prevalence of Zoonotic *I. ricinus*-Borne Pathogens in Natural Foci

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Environmental factors may drive tick ecology and therefore tick-borne pathogen (TBP) epidemiology, which determines the risk to animals and humans of becoming infected by TBPs. For this reason, the aim of this study was to analyze the influence of environmental factors on the abundance of immature-stage *Ixodes ricinus* ticks and on the prevalence of two zoonotic *I. ricinus*-borne pathogens in natural foci of endemicity. *I. ricinus* abundance was measured at nine sites in the northern Iberian Peninsula by dragging the vegetation with a cotton flannelette, and ungulate abundance was measured by means of dung counts. In addition to ungulate abundance, data on variables related to spatial location, climate, and soil were gathered from the study sites. *I. ricinus* adults, nymphs, and larvae were collected from the vegetation, and a representative subsample of *I. ricinus* nymphs from each study site was analyzed by PCR for the detection of *Borrelia burgdorferi* sensu lato and *Anaplasma phagocytophilum* DNA. Mean prevalences of these pathogens were 4.0% ± 1.8% and 20.5% ± 3.7%, respectively. Statistical analyses confirmed the influence of spatial factors, climate, and ungulate abundance on *I. ricinus* larva abundance, while nymph abundance was related only to climate. Interestingly, cattle abundance rather than deer abundance was the main driver of *B. burgdorferi* sensu lato and *A. phagocytophilum* prevalence in *I. ricinus* nymphs in the study sites, where both domestic and wild ungulates coexist. The increasing abundance of cattle seems to increase the risk of other hosts becoming infected by *A. phagocytophilum*, while reducing the risk of being infected by *B. burgdorferi* sensu lato. Controlling ticks in cattle in areas where they coexist with wild ungulates would be more effective for TBP control than reducing ungulate abundance.

Tick-borne zoonotic diseases are of great concern for public health authorities because of their increasing geographic range and the potential emergence of pathogens (35, 37, 72). Tick ecology (59) as well as other factors, such as climate, pathogen host community composition and density, habitat structure, and human activities (20, 27, 45, 46), greatly influences tick-borne pathogen (TBP) dynamics. Understanding TBP epidemiology and informing policy makers on TBP management require a basic knowledge of the regional and local factors influencing tick population dynamics, which constitutes the basis of this study.

The tick *Ixodes ricinus* is a three-host exophilic species that inhabits temperate and humid ecosystems of Europe, Asia, and northern Africa (23, 26) and is the most widely distributed and abundant tick species in Atlantic Iberia (8, 63). *I. ricinus* larvae feed mainly on small mammals, birds, and reptiles. Some larvae may be able to feed on the head and interdigital space of ungulates, where skin thickness is small enough to allow larval mouthparts to penetrate it (38). Nonetheless, ungulates are not considered main hosts for *I. ricinus* larvae (13, 30, 63). Nymphs feed on small and medium-sized mammals, birds, and large mammals, while adult ticks feed mainly on ungulates (24, 30, 53, 63, 66; but see reference 6). Ungulates are considered "tick reproduction hosts" (32), and immature host-seeking *I. ricinus* burdens are expected to be linked to the reproductive success of adult stages (65), although not necessarily in a linear way (the density of immature *I. ricinus* also depends on survival and feeding success). Hence, ungulates could potentially be targeted for the control of *I. ricinus* burdens, thus improving tick control efforts and reducing the associated costs.

Host individual features may influence tick population dynamics (7) and hence TBP epidemiology, but assessing their influence at the tick population scale is difficult. In addition to hosts, climate and habitat influence the global distribution of *I. ricinus* on a large scale (26) and its population dynamics at local and regional scales (8, 65). The variability in environmental factors between geographically close areas implies heterogeneity in the distribution and abundance of *I. ricinus* ticks in natural foci (7, 9, 22, 65), which means there is a heterogeneous risk of becoming infected by *I. ricinus*-borne zoonotic pathogens (36). The influence of small-scale climate and habitat (8, 22, 33, 54) and of host community composition and density (8, 74) on *I. ricinus* ecology has previously been investigated in Europe. Nonetheless, the effect of host abundance variability on *I. ricinus* abundance and pathogen epidemiology has scarcely been studied in Europe (but see references 62 and 65).

Among the wide range of tick-borne diseases present worldwide, Lyme borreliosis (LB) and human granulocytic anaplasmosis (HGA) are considered important threats to human and animal health (16, 34, 45, 51, 72). The causative agent of LB, *Borrelia burgdorferi* sensu lato, is a complex of bacteria responsible for clinical cases of Lyme disease in humans and animals. Lyme dis-

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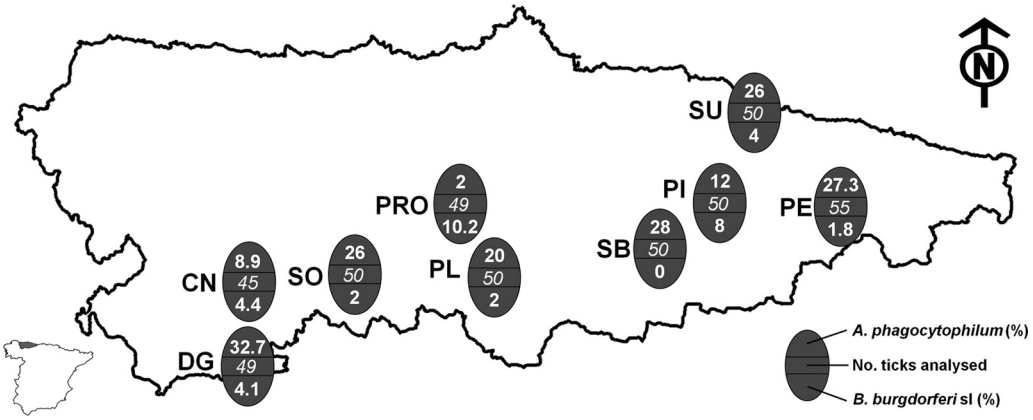

FIG 1 Map of the study region (Principado de Asturias, northern Spain) showing sampling sites, the number of *Ixodes ricinus* nymphs analyzed, and mean prevalence values for *Borrelia burgdorferi* sensu lato and *Anaplasma phagocytophilum* in *I. ricinus* nymphs.

ease is the most reported tick-borne pathogen in the Northern Hemisphere. Extensive literature is available on many aspects of *B. burgdorferi* sensu lato and LB (56). Nonetheless, data on the effects of the variability of local environmental factors on *B. burgdorferi* sensu lato epidemiology are scarce, especially in some of its southernmost distribution localities (but see references 8 and 25). Many vertebrate species, such as small mammals, birds, and reptiles, may act as reservoirs for *B. burgdorferi* sensu lato in natural biotopes (56), with *I. ricinus* being its main vector in Europe. *Anaplasma phagocytophilum* is an obligate intracellular bacterium that is distributed worldwide (18). *A. phagocytophilum* is the causative agent of HGA, tick-borne fever of ruminants, and equine and canine granulocytic anaplasmosis (21). *I. ricinus* is considered the main vector of *A. phagocytophilum* in Europe, although recent evidence suggests that other tick species may act as vectors in areas where *I. ricinus* is absent (15, 16). Small mammals and ungulates are deemed the most relevant reservoirs of *A. phagocytophilum* in natural foci, with persistent infections occurring in domestic and wild ruminants (17, 18, 27).

In this context, the main objective of this study was to investigate the effects of environmental factors on the abundance of immature *I. ricinus* ticks and on the epidemiology of two zoonotic *I. ricinus*-borne pathogens.

MATERIALS AND METHODS

Study area. Between 23 June and 3 July 2004, nine game preserves (CN, DG, PE, PI, PL, PRO, SB, SO, and SU) located in the Principado de Asturias autonomous community (northern Spain) were surveyed for ticks (Fig. 1). The studied areas are located within the Oceanic climate influence—a warm-temperate climate according to the Köpper-Geigen World Climate Classification map (39)—in the northern third of the Iberian Peninsula. These areas were selected as a subsample of the natural biotopes of Atlantic Iberia. A sampling site was selected from each game preserve as representative of the predominant habitats within the preserve. Study areas are characterized by regular year-round persistent rains (above 1,000 mm in a year) that decrease slightly during summer months. Habitat structure and composition may vary between sampling sites, but common features comprise variably sized deciduous forest patches with scattered natural or artificial grasslands, shrub, and scarce vegetation patches (for additional details, see reference 1).

Tick phenology varies according to tick developmental stage (i.e., larva, nymph, or adult), so sampling dates were selected to cover the host-seeking activity period of immature-stage *I. ricinus* ticks in areas of northern Spain (8, 9, 24; F. Ruiz-Fons, unpublished data). The survey was carried out on 11 consecutive days within the same season to allow for comparisons between sites, avoiding any confusion caused by variation in global weather conditions that could affect the host-seeking activity of ticks.

Tick data. The tick survey was performed by dragging the vegetation with a 2- by 1-m white cotton flannelette (31). Ten to 15 100-m drags were carried out per sampling site to estimate tick abundance according to previous studies (8, 67). Drags were stratified according to habitat structure by considering the cover percentage of the main grossly classified habitats (i.e., forest, shrub, scarce vegetation, and grasslands). In order to avoid the effect of changing weather conditions within a day, drags were performed at each site in 4 to 5 h in 1 day. Ticks were collected after each flannelette drag, stored in 70% ethanol, and transported to the laboratory, where they were identified (47). We calculated an abundance index for each stage of *I. ricinus* at the drag level as the number of ticks collected per 100 m.

Ecogeographical variables. To analyze the role of environmental variables on *I. ricinus* abundance and transmitted pathogen prevalence, statistical models were run using variables grouped into 4 factors: (i) spatial location, (ii) climate, (iii) soil, and (iv) hosts (Table 1). These ecogeographical variables were chosen on the basis of their availability at the scale of our study and of their known influence on the ecology of *I. ricinus* and TBP epidemiology (22, 24, 46, 59, 62, 65). Descriptive values of ecogeographical variables are summarized in Table 1.

(i) Spatial variables. Longitude and latitude at the centroid of each sampling site were recorded, since spatial data may reveal geographical trends in species distribution that can be associated with species population dynamics (44).

(ii) Climatic variables. Climate data were obtained from the work of Font (29). Details about how digital variables were obtained can be found in the work of Barbosa et al. (10). A detailed description of the climatic variables is given in Table 1. The average annual actual evapotranspiration (ETa) was considered a proxy of water saturation deficit (9). ETa is a measure of the water extracted from land surfaces due to evaporation and transpiration and hence a measure of the hydric stress conditions to which ticks are exposed (54).

(iii) Soil variables. Land cover variables (Table 1) were obtained from the CORINE Land Use/Land Cover database (28). Fortnight normalized difference vegetation index (NDVI) data for the period 26 June 2003 to 25 June 2004 were downloaded from the MODIS website (https://lpdaac.usgs.gov/lpdaac/get_data). NDVI seasonality was calculated as the variation coefficient of the fortnight measures for the year. Variables derived from the NDVI data were included within the soil factor (4), not within climate (57); even when it is indirectly related to precipitation, NDVI is a

TABLE 1 Ecogeographical variables (grouped into four factors) considered for modeling *Ixodes ricinus* abundance and *I. ricinus*-borne pathogen prevalence[a]

Factor	Variable code	Variable description	Mean (SE)	Range
Spatial location	Lo	Longitude (m)		
	La	Latitude (m)		
Climate	T	Avg annual mean temp (°C)	10.2 (1.6)	8.0–12.7
	Tr	Annual temp range (TJL − TJN) (°C)	13.0 (1.2)	11.4–14.9
	Fd	Avg no. of annual frost days	49.1 (30.0)	7.9–104.5
	P	Avg annual rainfall (mm)	1,248.3 (120.3)	1,028.0–1,344.3
	MP24	Avg maximum precipitation in 24 h (mm)	148.6 (20.9)	120.1–186.8
	MPr	Relative maximum precipitation (MP24/P)	0.09 (0.01)	0.07–0.12
	AHr	Annual air humidity range (HJL − HJN) (%)	9.1 (2.9)	3.4–12.1
	ETa	Avg annual actual evapotranspiration (mm)	648.4 (41.1)	596.8–725.8
Soil	F	Forest coverage (%)	0.25 (0.7)	0–2
	SV	Scarce vegetation coverage (%)	22 (12.4)	3–37
	G	Grassland coverage (%)	3.8 (6.0)	0–18
	NDVIa	Mean annual normalized difference vegetation index[b]	7,039.9 (374.7)	6,265.4–7,400.7
	NDVIc	Current NDVI (measured on 25 June 2004)[b]	8,404.3 (581.5)	7,280.9–9,098.4
	NDVIs	NDVI seasonality	0.17 (0.07)	0.07–0.27
Hosts	Cab	Cattle abundance index (no. of 10-m transects positive for host dung in 100 m)	0.37 (0.03)	0–0.60
	Hab	Horse abundance index (no. of 10-m transects positive for host dung in 100 m)	0.03 (0.01)	0–0.23
	Dab	Deer abundance index (no. of 10-m transects positive for host dung in 100 m)	0.05 (0.01)	0–0.27

[a] The table shows the mean value for each variable at the study sites (except for site PI) and its associated standard error (SE) as well as the observed range (minimum to maximum) of values for each variable at the surveyed sites. TJL, average mean temperature in July; TJN, average mean temperature in January; HJL, average air humidity range in July; HJN, average air humidity range in January.

[b] NDVI values were divided by a scale factor of 0.0001.

measure of the amount and vigor of vegetation on the land surface related to soil moisture (49).

(iv) Host abundance. An abundance index for the most abundant ungulates (cattle, horse, and deer) in the studied areas was calculated. For this index, ungulate dung presence was recorded for a 2-m wide band at 10-m intervals during walking transects carried out in parallel to blanket drags. The abundance index per sampling site was calculated—for each species—as the mean number of 10-m transects positive for host dung in 100 m (2). Horse and cattle dung presence was considered when at least one fecal pellet was found within the 10-m-long interval. A 10-m interval was considered positive for deer (*Cervus elaphus*, *Capreolus capreolus*, or *Dama dama*) dung presence if a group of at least 6 pellets was located within the transect band (12).

The spatial resolution of the variables was not homogeneous between factors, and thus all the ecogeographical information was finally translated into 10- by 10-km UTM squares by use of the Extract module of the Idrisi Andes software package. Every flannelette drag was georeferenced with a portable GPS device and then assigned to one UTM square for characterizing the ecogeographical variables. One 10- by 10-km UTM square included all drags carried out within a given preserve.

Molecular analyses of tick-borne pathogens. Similar numbers of *I. ricinus* nymphs by preserve (Fig. 1) were used for DNA extraction by use of a DNeasy blood and tissue kit (Qiagen, Hilden, Germany) according to the manufacturer's instructions. The presence of *B. burgdorferi* sensu lato DNA was detected using a nested PCR approach (61). Two sets of primers were used to amplify a fragment of the spacer region between the 5S and 23S rRNA genes of *B. burgdorferi* sensu lato. The outer primers were 23SN1 (5′-ACCATAGACTCTTATTACTTTGAC-3′) and 23SC1 (5′-TAAGCTGACTAATACTAATTACCC-3′), and the nested primers were 23SN2 (5′-ACCATAGACTCTTATTACTTTGACCA-3′) and 5SCB (5′-GAGAGTAGGTTATTGCCAGGG-3′). The outer primers produced a fragment of 380 bp, and the nested primers produced a fragment of approximately 225 bp, depending on the *Borrelia* genospecies amplified.

In the case of *A. phagocytophilum*, 16S rRNA gene sequences were amplified by PCR as reported previously (16, 17), using species-specific oligonucleotide primers 16SANA-F (5′-CAGAGTTTGATCCTGGCTCAGAACG-3′) and 16SANA-R (5′-GAGTTTGCCGGGACTTCTTCTGTA-3′), which produced a fragment of 468 bp as described previously (16, 17, 69). The standardized PCR mix consisted of 1 μl (1 to 10 ng) DNA with 10 pmol of each primer in a 50-ml PCR mix (1.5 mM $MgSO_4$, a 0.2 mM concentration of each deoxynucleoside triphosphate [dNTP], 1× avian myeloblastosis virus [AMV]/*Tfl* reaction buffer, 5 U *Tfl* DNA polymerase), and PCR was performed by employing an Access RT-PCR system (Promega, Madison, WI). In the case of the nested PCR for *B. burgdorferi* sensu lato, 5 μl of product from the first PCR was added to 45 μl of the second PCR mix. Reactions were performed in an automated DNA thermal cycler (Techne TC-512; Durviz, Valencia, Spain). The PCR conditions for *B. burgdorferi* sensu lato were the following. For the first PCR, the samples were denatured at 94.5°C for 60 s, followed by 30 cycles of 20 s at 94°C, 20 s at 52°C, and 45 s at 68°C, with a final extension step of 5 min at 68°C. For the second PCR, a denaturation step at 94.5°C for 60 s was followed by 40 cycles of 20 s at 94°C, 20 s at 52°C, and 55 s at 68°C, with a final extension step of 5 min at 68°C. In the case of *A. phagocytophilum*, PCR conditions were the same as those reported previously (16, 17, 69). PCR products were electrophoresed in 1% agarose gels to check the sizes of amplified fragments by comparison to a DNA molecular size marker (1 kb Plus DNA ladder; Promega).

Amplicons were cloned into pGEM-T (Promega, Madison, WI), and 3 (in the case of *B. burgdorferi* sensu lato) or 6 (in the case of *A. phagocytophilum*) independent clones were sequenced from both ends for each gene marker. Sequence similarity searches were performed by using BLAST (http://www.ncbi.nlm.nih.gov).

Statistical analyses. The influence of environmental factors on immature-stage *I. ricinus* abundance was tested by using generalized linear models (GLM), with *I. ricinus* abundance indices at the drag level as response variables and the covariables shown in Table 1 as predictors. A preliminary variable reduction process was carried out to avoid multicolinearity (e.g., see reference 64) by generating a correlation matrix for each

TABLE 2 Mean questing *Ixodes ricinus* larva, nymph, and adult abundance indices per sampling site and for the global study area

Sampling site	Mean abundance index (no. of ticks/100 m)		
	Larva	Nymph	Adult
CN	0.0	5.9	0.2
DG	2.1	6.8	0.1
PE	73.8	19.5	0.3
PI[a]	14.4	68.4	1.1
PL	3.3	4.8	0.2
PRO	28.1	5.3	0.0
SB	82.6	27.4	0.4
SO	27.9	93.6	2.0
SU	165.3	7.8	0.0
Total	42.0	22.4	0.4

[a] Site PI was not considered for statistical modeling.

of the ecogeographical factors considered. After that, every one of the highly correlated variables (rho $\geq |0.7|$) within each factor was tested against the response variable by the Spearman correlation test. Finally, only the predictor variable from each set of highly correlated variables that was better correlated with the response variable was retained to be included in the model. Models were performed separately for each *I. ricinus* immature stage, since the scarcity of capture of adult *I. ricinus* ticks (Table 2) did not allow for testing of the effect of ecogeographical variables on adult tick abundance. Site PI was not considered in the models because of a logistic constraint that altered the record of host abundance. Due to the aggregation of tick abundance data, models were fitted with a negative binomial probability distribution and a logarithmic link function. We used a forward stepwise procedure using the Akaike information criterion (AIC) (5) to select the most parsimonious model. Thus, to build the minimal adequate model, we first fitted univariate models and retained that with the lowest AIC. We then continued to test bivariate models, always retaining the one with the lowest absolute AIC. We finished the procedure when it was not possible to reduce the AIC value by adding a new variable to the model, as described elsewhere (e.g., see references 3 and 64).

Thereafter, GLM (binomial distribution and logistic link function) was run using the presence/absence of *B. burgdorferi* sensu lato or *A. phagocytophilum* at the *I. ricinus* nymph level as a response variable. Site PI was again not considered in the models. The same fixed environmental predictors considered in the tick abundance analyses were considered herein, as they were also better correlated with pathogen presence. Modeling was carried out as described for the above-mentioned tick abundance analyses, that is, by using a forward stepwise procedure based on the AIC.

In testing statistical hypotheses by standard methods (e.g., GLM), standard errors are usually underestimated when positive autocorrelation is present, and consequently, type I errors—i.e., rejecting a true null hypothesis—may be inflated strongly (44). In order to account for this potential bias in the models, residuals of both tick and pathogen final models were examined and tested for autocorrelation using Moran's I statistic (see reference 19). Moran's I test was checked for significance with the Bonferroni-corrected significance level.

RESULTS

Tick abundance. Globally, 2,912 *I. ricinus* larvae, 1,846 nymphs, and 29 adults were collected in the 9 surveyed preserves. The tick capture ratio (larva:nymph:adult) was 100.4:63.7:1. A detailed report of the questing tick abundance index is provided in Table 2.

Results of the final model for larva abundance showed a statistically significant influence of longitude and latitude (Table 3). These results evidenced a positive east- and northward increasing trend in larva abundance, with the abundance being higher at sites close to the coastal border (Fig. 1; Table 2). Model results also showed a statistically significant positive influence of increasing mean annual temperature and a negative effect of increasing mean annual number of frost days on the abundance of *I. ricinus* larvae (Table 3). This means that there was a higher abundance of larvae at sites with a higher mean annual temperature and a lower mean annual number of days with minimum temperatures below the freezing point. Finally, the only variable in the host factor that showed a statistically significant relationship with larva abundance was horse abundance (Table 3): larvae were more abundant at those sites with a higher horse abundance.

The final model output for nymph abundance showed that burdens of *I. ricinus* nymphs were statistically significantly positively related to moisture variables such as annual rainfall, maximum precipitation in 24 h, and the NDVI during the period of sampling (Table 3). Model results also evidenced that increasing mean annual temperature also had a statistically significant positive influence on *I. ricinus* nymph abundance. The only statistically significant environmental variable found to negatively influence nymph abundance was ETa (Table 3); nymph abundance was lower at sites where the hydric stress was higher.

No statistically significant Moran's I value was observed in ex-

TABLE 3 - Model estimates (*B* coefficient and its associated standard error [SE]), Wald chi-square statistic values, and *P* values for final generalized linear models (negative binomial probability distribution and logarithmic link function) of *Ixodes ricinus* abundance[a]

Tick stage	Variable	*B* coefficient	SE	Wald χ^2 value	*P* value
Larva	Intercept	−1,394.0	389.5	12.8	<0.001
	Lo	4.6×10^{-5}	6.3×10^{-6}	52.1	<0.001
	T	0.8	0.2	20.3	<0.001
	Fd	−0.2	3.0×10^{-2}	29.7	<0.001
	La	0.1×10^{-4}	8.1×10^{-5}	12.3	<0.001
	Hab	8.8	4.1	4.6	<0.05
Nymph	Intercept	−33.1	9.6	11.9	<0.01
	ETa	−0.1	5.2×10^{-3}	5.7	<0.05
	P	4.0×10^{-3}	1.3×10^{-3}	10.2	<0.01
	T	1.0	0.2	37.6	<0.001
	MP24	9.0×10^{-2}	1.9×10^{-2}	21.4	<0.001
	NDVIc	2.0×10^{-3}	5.0×10^{-4}	12.8	<0.001

[a] Model outputs are shown separately for the different immature stages of *I. ricinus*. Variable abbreviations are described in Table 1.

TABLE 4 Model estimates (*B* coefficient and its associated standard error [SE]), Wald chi-square statistic values, and *P* values for final generalized linear models (binomial distribution and logistic link function) of *Ixodes ricinus*-borne pathogens (*Borrelia burgdorferi* sensu lato and *Anaplasma phagocytophilum*)[a]

Pathogen	Variable	*B* coefficient	SE	Wald χ^2 value	*P* value
B. burgdorferi sensu lato	Intercept	−2.6	0.4	3.4	<0.05
	Cab	−3.6	1.4	6.8	<0.05
	G	0.1	5.7×10^{-2}	2.7	0.1
A. phagocytophilum	Intercept	−3.9	0.6	9.1	<0.001
	Cab	3.6	0.9	16.7	<0.001
	Fd	1.6×10^{-2}	5×10^{-3}	10.7	<0.01

[a] Models were carried out in a separate way for each pathogen. Variable abbreviations are described in Table 1.

ploring the Pearson's residuals of each model. The indices attained very low values (average Moran's I values for 10 distance classes were −0.008 and 0.009 for larva and nymph models, respectively), indicating the inexistence of a solid spatial structure of the residuals, which suggests no problems related to type I errors and also that the most relevant spatially structured predictors were included in the models (see reference 75).

Pathogen prevalence. A representative subsample of *I. ricinus* nymphs from each sampling site (Fig. 1) was tested for the presence of *B. burgdorferi* sensu lato and *A. phagocytophilum* DNA. A total of 4.0% ± 1.8% and 20.5% ± 3.7% of the analyzed nymphs were positive for *B. burgdorferi* sensu lato and *A. phagocytophilum* DNA, respectively. Prevalence values ranged from 0% to 10.2% for *B. burgdorferi* sensu lato and from 2% to 32.7% for *A. phagocytophilum* (Fig. 1). Interestingly, a statistically significant negative relationship ($B = -2.5$; $R^2 = 0.597$; $P < 0.05$; $n = 9$) was evidenced between *B. burgdorferi* sensu lato and *A. phagocytophilum* prevalences at the study site level.

The results of the final model for *B. burgdorferi* sensu lato showed that the risk of *I. ricinus* nymphs carrying *B. burgdorferi* sensu lato DNA was statistically significantly related to cattle abundance. The observed negative relationship between these variables suggests a lower *B. burgdorferi* sensu lato prevalence at sites with a higher cattle abundance (Table 4). Regarding *A. phagocytophilum*, the final model evidenced statistically significant relationships between the mean annual number of frost days and cattle abundance and the risk of *I. ricinus* nymphs carrying *A. phagocytophilum* DNA. No statistically significant Moran's I value was found in exploring the Pearson's residuals of these models. The indices attained very low values (average Moran's I values for 17 distance classes were −0.002 and −0.009 for *B. burgdorferi* sensu lato and *A. phagocytophilum* models, respectively).

DISCUSSION

Research efforts in this study were focused on the most abundant ixodid tick species in Atlantic Iberia, i.e, *I. ricinus*, and on two of the most relevant tick-borne pathogens in Europe from a human and animal health perspective, i.e., *B. burgdorferi* sensu lato and *A. phagocytophilum*. While spatial location and climate factors were the main environmental drivers of *I. ricinus* abundance, host abundance was the main factor influencing the risk of nymphs being infected by *B. burgdorferi* sensu lato and *A. phagocytophilum*. Ungulates are known drivers of TBP, such as louping ill virus (30, 43) and tick-borne encephalitis virus (62), and cattle specifically have been linked previously to the risk of nymphs carrying *B. burgdorferi* (60).

Methodological considerations. A survey of tick population abundance during a short period within a year may impair the representativeness of model results if it is not carried out in an appropriate window of host-seeking activity for the different tick stages. This study was planned according to the expected window of activity of *I. ricinus* larvae and nymphs in northern Spain (24). The activity period of *I. ricinus* ticks in northern Spain is slightly (larvae) to moderately (nymphs) bimodal, with overlapping activity of both stages in early summer (8, 24). The main peaks of host-seeking activity of immature-stage *I. ricinus* ticks were reported to be similar between areas with differing climate influences—Oceanic versus Mediterranean climates—in the northern Iberian Peninsula (8, 24). According to this observation, variation in the main questing activity season of immature-stage *I. ricinus* ticks at the study sites—all of them under the influence of an Oceanic climate—would be expected to be low. Additionally, the questing activity of all stages of *I. ricinus* ticks in southern England and Ireland (under moderate Oceanic climate influence, like the Principado de Asturias) shows a constant spring-to-summer activity (42). Thus, although long-term series data on tick abundance are desirable for tick ecology studies, punctual well-balanced surveys carried out within the activity period of different tick stages may also be representative of tick abundance at regional and local scales (see reference 65).

A combination of long-term and short-term data on the most relevant climatic determinants of *I. ricinus* abundance was used. The long-term series climate data employed for modeling purposes were an interpolation of climatic data from meteorological stations (29) and were thus a more accurate measure of the climatic conditions of our study sites because these data were available at high spatial resolution. Short-term climatic variation, e.g., an extremely cold winter or drought, may condition tick survival and hence tick abundance, so combining long-term and short-term climate data in this situation is desirable. Short-term climate data were not available at the study scale, except for the NDVI, which was used to control for short-term climatic variations between our study sites. The NDVI is related to soil moisture, an environmental feature conditioning *I. ricinus* survival (32).

Environmental determinants of tick abundance. Host-seeking *I. ricinus* larva abundance is expected to be a consequence of a higher reproductive success of adult ticks (see reference 68), although not necessarily in a linear fashion, due to the influence of environmental factors on larva survival (54). Model results showed the influence of horse abundance on larva abundance. However, horses were present at only the two easternmost sites

(SU and PE). Since larvae were predicted to be more abundant at northeastern sites due to temperature, this may have been the cause for the observed effect of horse abundance. This agrees with observations in the Scottish Highlands, where adult *I. ricinus* hosts scarcely influenced the abundance of larvae (65), but contrasts with the influence of adult *Ixodes scapularis* hosts on the abundance of immature-stage ticks at microgeographic scales (73). The effect of temperature-related variables on *I. ricinus* larva abundance suggests that survival of larvae can be conditioned by winter severity. Thus, when moisture deficit is not an important stressing factor (see the standard deviation for ETa in Table 1), the abundance of ticks may be determined largely by the severity of the winter season. From a tick and TBP control point of view, this finding could help in planning annual control efforts depending on the winter conditions, thus reducing the associated costs. This finding is additionally supported by the observed increasing trend in larva abundance on a southwestern-northeastern axis. Both the longitude and latitude of the studied areas were positively correlated with mean annual temperature and negatively correlated with the annual number of frost days (data not shown), showing a clear influence of the buffering climatic effect of the sea on small-scale climatic conditions. Contrasting results with those of studies in the Scottish Highlands (65) could be associated with the colder winter weather in northern Scotland, which would reduce larva survival in comparison to that in northern Spain.

In contrast to larva abundance, nymph abundance is expected to be modulated mainly (when speaking about host influence) by larva host abundance, i.e., the abundance of small mammals, birds, and reptiles. However, there is evidence of adult tick host abundance influencing nymph abundance in the Scottish Highlands (65), which is thought to be an effect of ungulates acting as tick reproduction hosts (32). The final model for nymphs revealed no significant influence of ungulate abundance on nymph abundance over the rest of the ecogeographical variables considered. The feeding success of *I. ricinus* larvae, which is dependent on the abundance of appropriate hosts, may be a better predictor of nymph abundance than ungulate abundance. Unfortunately, logistic constraints did not allow for measurements of small mammal and bird abundances and for testing of their influence on nymph abundance. Nymphs are expected to be more tolerant to hydric stress than larvae, but the former were more abundant than the latter at sites with higher humidity. This could perhaps be related to differences in questing behavior; while larvae quest low in the ground vegetation looking for hosts, nymphs quest higher and are more exposed to water loss. However, both larva and nymph abundances were positively influenced by increasing mean temperature, which means there was an expected higher abundance in coastal than in inner areas of the study region.

Epidemiologic drivers of *B. burgdorferi* sensu lato and *A. phagocytophilum* in questing nymphs. The most important competent animal reservoirs of *B. burgdorferi* sensu lato in Europe are rodents such as *Apodemus* sp. mice and voles, insectivores such as shrews and hedgehogs, lagomorphs such as hares, and several species of resident and migratory birds (14, 40, 48, 70). Ungulates are known incompetent reservoirs of *B. burgdorferi* sensu lato (41, 48). Thus, the higher the abundance of ungulates, the lower is the expected prevalence of *B. burgdorferi* sensu lato in ticks, without any additional information on persistence of *B. burgdorferi* sensu lato in natural foci of endemicity. The observed dilution effect of cattle abundance on the prevalence of *B. burgdorferi* sensu lato apparently contradicts the consideration of ungulates as passive reservoirs allowing tick cofeeding transmission, as reported for sheep (50). However, cattle are not among the preferred hosts of immature-stage *I. ricinus* ticks, and hence successful cofeeding transmission of *B. burgdorferi* sensu lato may not be common in cattle. The role of ungulates as *I. ricinus* reproduction hosts may determine higher immature tick burdens (i.e., larvae), but with an apparently lower risk to humans of being bitten by a *B. burgdorferi* sensu lato-infected *I. ricinus* nymph. However, the abundance of adult *I. ricinus* hosts was found to be linked to the number of LB cases reported in humans (74), which may suggest that the risk is maintained in spite of the dilution effect of ungulates.

In contrast to the case with *B. burgdorferi* sensu lato, many ungulate species are competent reservoirs of *A. phagocytophilum* (16). Climate was also found to be a relevant driver of *A. phagocytophilum* prevalence in *I. ricinus* nymphs from Atlantic Iberia. The positive effect of the mean annual number of frost days on prevalence may underlie a higher diversity of *A. phagocytophilum* reservoir hosts, because inner areas in the region—which are slightly colder than coastal areas—are less influenced by human activities. It was surprising that deer abundance was not included in the final model. Red and roe deer are thought to be greatly relevant in the wild cycle of *A. phagocytophilum* (55). The higher abundance of cattle than deer at our study sites may have undermined the effect of deer on the prevalence of *A. phagocytophilum*, which may be driven by wild ungulates in areas of livestock absence. This study concerned only the cycle of *A. phagocytophilum* involving exophilic ticks and hosts. Nonetheless, Bown et al. (11) confirmed that wild wood rodents may maintain *A. phagocytophilum per se* in forested biotopes in the United Kingdom, which may imply that endophilic and exophilic cycles coexist and link to drive *A. phagocytophilum* ecology.

Implications of our results for tick and TBP control schemes. Since promoting basic epidemiological information on TBP is paramount for the implementation of efficient and environmentally friendly control measures, targeted control efforts may significantly improve cost efficiency and reduce unnecessary environmental pollution. Ungulates are relevant drivers of TBP prevalence, which suggests that they may be adequate targets for controlling TBP in oceanic climate areas of the Iberian Peninsula. Nonetheless, in looking at the environmental factors driving *I. ricinus* nymph abundance, one may think that strategies based on reducing ungulate abundance may not be effective enough to reduce nymph abundance, the main target for TBP control. Additionally, reducing ungulate abundance may differently affect the prevalence of nymphs infected by *B. burgdorferi* sensu lato and *A. phagocytophilum*. These facts suggest that tick control measures in hosts—as the measures themselves or within an integrated tick control scheme—may be more efficient than reducing the abundance of adult *I. ricinus* hosts. Thus, tick control measures in Atlantic Iberia should target cattle where they coexist with wild ungulates. Further research on the feasibility and efficiency of tick control measures on wild ungulates in Atlantic Iberia is needed to reduce the risk of TBP transmission in natural areas. The influence of small-scale climatic conditions on tick abundance suggests that tick control efforts should be applied differently to areas with high and low tick abundances.

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The authors declare that they have no conflict of interest.

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